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Biodiversity of heat-resistant ascomycetes from semi-arid soils in Argentina

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Abstract—Artificial thermal shock conducted on 50 soil samples from a semi-arid geographic region (Catamarca and La Rioja provinces) in northern Argentina yielded 34 heat-resistant fungal strains. These strains were assigned to seventeen taxa in ten ascomycete genera: *Arthrinium* (1), *Aspergillus* (3), *Epicoccum* (1), *Gilmaniella* (1), *Hamigera* (2), *Leiothecium* (1), *Penicillium* (2), *Talaromyces* (4), *Trichocladium* (1), and *Trichoderma* (1). All strains were identified by phenotypic features, with molecular data additionally obtained for eleven strains. Five species are reported for the first time in Argentina.

Key words —*Ascomycota*, *Eurotiales*, South America, Monte, Chaco.

Introduction

Temperature is an environmental parameter that plays a key role in the survival, growth, distribution, and speciation of microorganisms on earth (Mouchacca 1993). Fungi growing at high temperatures are generally classified

in two groups: thermophilic and thermotolerant. Thermophilic fungi are those able to grow in a temperature range of 20–50 °C or above; while the maximum temperature for growth of thermotolerant fungi is near 50 °C with a minimum of below 20 °C (Cooney & Emerson 1964, Mouchacca 2007).

Alternatively, heat-resistant fungi are defined as those capable of surviving to a thermal shock, e.g., temperatures at or above 75 °C for 30 minutes or more (Samson & al. 2000). The fungal structures able to survive these extreme temperatures are mainly ascospores, but other propagules such as chlamydospores, thick-walled hyphae, and sclerotia, also may survive such temperature extreme (Scholte & al. 2004). Dijksterhuis & Samson (2006) expanded the concept of heat-resistant fungi to include most of fungal structures that survive after a thermal shock between 55–95 °C. Heat resistance is associated with the ability to maintain the viability of certain fungal structures in order to overcome natural thermal shock. Thus, heat-resistant fungi can be mesophilic, thermotolerant, or thermophilic.

Soil, the primary reservoir of fungi, is also the main repository of dormant propagules of true soil-borne fungi as well as of fungi that grow elsewhere. Most fungi achieve survival through the production of both dormant and dispersal spores (Carlile & Watkinson 1994). Latent spores normally germinate due to slow dormancy decay or in response to a specific (physical or chemical) stimulus that breaks the dormancy (Carlile & Watkinson 1994), such as a thermal shock. Constitutive dormancy is a condition in which the development is delayed by an innate property, such as a barrier to the penetration of nutrients (including water), a metabolic blockage, or the action of a self-inhibitory molecule (Dijksterhuis & Samson 2006, Dijksterhuis 2007). Ascospores, the sexual propagules of the *Ascomycota,* often have a long survival capability (Dijksterhuis 2007). Ascospores of heat-resistant fungi exhibit constitutive dormancy and require a robust signal (heat, high pressure, or exposure to organic compounds, such as dimethylketone) to break the dormancy (Dijksterhuis 2007, Stchigel 2000). Warcup & Baker (1963) noted an increase in the number of ascomycete colonies when soil samples were exposed to temperatures between 50–75 °C for 30 minutes before culturing, compared with those not exposed to this thermal shock.

The fungal soil communities from arid regions are mainly characterized by a low population coupled with a high biodiversity (Mouchacca 1993). During broad research on heat resistant fungi from the semi-arid region of Northern Argentina 194 isolates were obtained. Most isolates were related to *Aspergillus* sect. *Fumigati,* but 34 isolates represented different

interesting taxa. This study documents those 34 miscellaneous heat-resistant ascomycetes isolated from soil.

Materials & methods

Description of the sampling area

Fifty 200-g soil samples were collected in the summer of 2009 and winter of 2011 from several sites in Catamarca and La Rioja provinces, Northern Argentina. Of the six phytogeographical regions of Catamarca (Morláns 1995), most samples were collected from the "monte" region and only a few from the "chaqueña" region; additionally three collections were sampled from the "monte" region of La Rioja (with five phytogeographical regions). The monte region in Catamarca, which comprises c. 14,600 km^2 (Manghi & al. 2005) and extends through the center of La Rioja province, is characterized by steppe vegetation dominated by xerophilous, psammophilous, or halophilous shrubs, and characterized by the almost constant presence of *Larrea* (*Zygophyllaceae*) and *Prosopis* (*Leguminosae*). The climax community is *Larrea divaricata* Cav. ("jarillal"), which develops in the sandy pockets and plains or stony-sandy soils (Cabrera 1971). Climate in the monte is subtropical, arid, with an annual 150–200 mm precipitation occurring mostly in the summer (60–70% of total rainfall; Morláns 1995). Temperature shows a relatively broad seasonal and daily variation, with autumn/winter lows (–30 °C in west mountain region) and spring/summer highs (45 °C in the east and central regions) (Gobierno de Catamarca 2018, Morláns 1995). The strong winds throughout the year favor environmental dryness. The monte elevation ranges from 600–3000 m a.s.l. (Morláns 1995).

Fungal isolation

From each sample, c. 5 g of soil was placed into a conical flask, mixed with 100 ml of sterile melted malt extract agar (Oxoid MEA: malt extract 30.0 g, mycological peptone 5.0 g, agar 15.0 g) plus 50 ppm of chloramphenicol, and heated at 75 °C for 30 min. The suspension was plated into sterile Petri dishes and, once gelled, incubated at 30 °C ≤30 d (Samson & al. 2000). All colonies were transferred to individual Petri dishes containing MEA to obtain pure cultures. The fungal strains were deposited in the culture collection of the Herbarium, Universidad de Buenos Aires, Argentina (BAFC) and the culture collection of Faculty of Medicine, Universitat Rovira i Virgili, Reus, Catalonia, Spain (FMR). Author abbreviations include SMR (S.M. Romero), AIR (A.I. Romero), AMS (Stchigel), ERA (Rodríguez Andrade), VB (Barrera), and RC (Comerio). Abbreviations under Culture(s) examined are RN (Ruta Nacional) and RP (Ruta Provincial).

Morphological study

Preliminary characterization at the genus level was conducted on MEA (Domsch & al. 2007, Guarro & al. 2012). Species were identified by growing the fungal strains on different culture media and temperatures (following the bibliography for each genus mentioned in each description). Vegetative and reproductive structures were

microscopically examined by mounting directly into lactic acid from cultures on MEA and then observed under a Zeiss Axioskop bright field microscope. Mature ascomata were crushed, coated with gold, and observed and photographed with a Zeiss Supra 40 (extra high tension = 3 Kv; working distance = 3.7–6 mm) scanning electron microscope.

Molecular study

Extraction: DNA was extracted directly from colonies on potato dextrose agar (PDA) after 7–10 d at 25 °C in darkness, through the modified protocol of Müller & al. (1998). The following genetic markers were sequenced: fragments of ß-tubulin (BenA), calmodulin (CaM), RNA polymerase II second largest subunit (rpb2) (Peterson & al. 2010, Samson & al. 2014), and the DNA replication licensing factor (Mcm7) (Schmitt & al. 2009, Raja & al. 2011). The primers used were Bt2a and Bt2b for BenA, CMD5 and CMD6 for CaM (Glass & Donaldson 1995), RPB2-5F and RPB2-7R for rpb2 (Liu & al. 1999), and Mcm7-709For and Mcm7-1348Rev for Mcm7 (Schmitt & al. 2009). PCR products were sequenced in both directions using the same primers at Macrogen Europe (Macrogen Inc., Madrid, Spain). Sequences were assembled and edited using Sequencher v.4.1.4. The sequences generated were deposited in the GenBank database (https:// www.ncbi.nlm.nih.gov/genbank) using the Webin platform of the European Bioinformatics Institute (EMBL-EBI) (http://www.ebi.ac.uk/ena).

Phylogenetic analysis: Multiple sequence alignment was conducted using ClustalW (Thompson & al. 1994) and MUSCLE (Edgar 2004) within MEGA v.6 software (Tamura & al. 2013), with manual adjustments for refinement. The Maximum Likelihood (ML) phylogenetic method was also run with MEGA v.6, as well as the estimation of the best nucleotide substitution model. Support of the internal branches was assessed by the bootstrap method with 1000 replications, where values ≥70 were considered significant. The phylogenetic analyses were carried out first individually for each gene followed by a concatenated study. To support our analyses, sequences were obtained from GenBank and added to the analyses.

Taxonomy

In our study, 34 heat-resistant fungal strains from the semiarid region in Argentina were all morphologically characterized and assigned to 17 taxa in 10 genera—*Arthrinium* (1), *Aspergillus* (3), *Epicoccum* (1), *Gilmaniella* (1), *Hamigera* (2), *Leiothecium* (1), *Penicillium* (2), *Talaromyces* (4), *Trichocladium* (1), and *Trichoderma* (1). For 11 strains, the morphological identification was confirmed by molecular analyses. Five taxa are reported for the first time in Argentina.

Arthrinium phaeospermum (Corda) M.B. Ellis, Mycol. Pap. 103: 8. 1965.

Colonies on MEA, 25 °C, 7 d, floccose, covering the whole culture plate, greyish, with dark specks and areas due to the anamorph presence.

Conidia lenticular, dark brown, 9–11 × 5–7 µm, smooth with a conspicuous pale equatorial stripe.

Cultures examined—**ARGENTINA. Catamarca: RP 46**, km 70, 28°05′35″S 66°12′55″W, 859 m a.s.l., 24.VIII.2011, leg. SMR, identified by SMR & RC, isolation 22.X.2011 (BAFCcult 4624); 27°35′13″S 66°22′11″W, 996 m a.s.l., 24.VIII.2011, leg. SMR, identified by SMR & RC, isolation 15.XI.2011 (BAFCcult 4625).

Distribution—Worldwide, including Argentina (Broggi & al. 2007, Crous & Groenewald 2013, Domsch & al. 2007).

Habitat—Cereals and vegetable materials, dung, fresh water, and soil (Domsch & al. 2007).

Comments—Our isolates agreed morphologically with descriptions by Ellis (1971), Domsch & al. (2007), and Crous & Groenewald (2013). In Argentina, this species was isolated from soil in Buenos Aires (Cabello 1986) and cereals collected in Entre Ríos (Broggi & al. 2007, Sacchi & al. 2009).

Aspergillus flavus Link, Mag. Ges. Naturf. Freunde, Berlin 3: 16. 1809.

Colonies on MEA, 25 °C, 7 d, plane, granulose, floccose at the centre, 50–54 mm diam., yellowish green, exudate scarce uncolored; asexual sporulation abundant. On Czapek yeast agar (CYA), 25 °C, 7 d, sulcate, granulose, floccose at the centre, 60-68 mm diam., yellowish green, exudate scarce uncolored; asexual sporulation abundant.

Conidial heads commonly columnar, radiate, mainly uniseriate. Stipes roughened, vesicles spherical. Conidia globose, 3.5–4.5 µm, with finely roughened walls.

Culture examined—**ARGENTINA. La Rioja: RN 60**, km 1172, 28°40′30″S 66°30′23″W, 653 m a.s.l., 12.I.2009, leg. SMR, identified by SMR, isolation 1.III.2011 (BAFCcult 4615).

Distribution—Worldwide and cosmopolitan (Domsch & al. 2007; Ramírez-Camejo & al. 2012).

Habitat—Soil, cultivated or not (Domsch & al. 2007). This is the most widely cited fungal species as a food contaminant (Klich 2002, Pitt & Hocking 2009).

Comments—The micromorphology of our isolate, and even its cultural features, agreed with the descriptions of Klich (2002) and Pitt & Hocking (2009). Horn & al. (2009) described the sexual stage for this heterothallic species as *Petromyces flavus* B.W. Horn & al., but according to the International Code of Nomenclature (Turland & al. 2018) and following Samson & al. (2014), the correct name for this species is *Aspergillus flavus*. In Argentina,

it has been isolated several times from soil (Allegrucci & al. 2009, Cabello 1986, Godeas & al. 1977, Mangiaterra & al. 2006, Winitzky 1948), foods (Bresler & al. 1991, Castellari & al. 2015, Dalcero & al. 1998, Etcheverry & al. 1999, Romero & al. 2005, Sepúlveda & Piontelli 2005) and medicinal herbs (Sánchez & al. 2006).

Aspergillus montevidensis Talice & J.A. Mackinnon,
C.r. Seanc. Soc. Biol. 108: 1007. 1931. Fig. 1a,b

Colonies on MEA, 25 °C, 7 d, plane and dense, 14 mm diam., yellowish; after 14 incubation d 26–28 mm diam., yellowish, with or without brown center. On Czapek yeast agar with 20% sucrose, 25 °C, 7 d, velutinous, 35–47 mm diam., yellow, with green center due to the anamorph presence; after 14 incubation d covering the whole culture plate, velutinous to granulose due to the abundant cleistothecia production, yellow with green center; asexual sporulation copious; reverse yellowish under cleistothecia and greenish under anamorph structures. At 37 °C, 7 d, 18–26 mm diam., yellow.

Cleistothecia superficial, mainly globose, discrete, 90–150 µm diam., yellow, covered by a hyaline hyphal net. Asci globose, 8-spored, 10–12 × 8–11 µm, evanescent. Ascospores lenticular, 5–4 × 3–4 µm, with rough convex surfaces, furrowed and double-crested along the equator; SEM images revealed pores distributed in the whole ascospore surface, but mainly on crests. Conidial heads radiate, uniseriate. stipes 150–210 × 6–8 µm, smooth, vesicles globose, 15–27 µm diam. Phialides ampulliform, 5–8 × 4 µm. Conidia globose to subglobose, 5–4 × 4–3 µm, spinulose.

Cultures examined—**ARGENTINA. Catamarca**: **RN 46**, km 185, 27°45'12"S 66°47'59"W, 1069 m a.s.l.. 9.I.2009, leg. SMR, identified by SMR, isolation 21.III.2011 (BAFCcult 4613; GenBank LT964777, LT964778); **Papachacra**, 2564 m a.s.l., 10.I.2009, leg. SMR, identified by SMR, isolation 1.III.2011 (BAFCcult 4614; GenBank LT964779, LT964780).

Distribution—Argentina (Winitzky 1952), Australia, Brazil, India, Israel, Japan, Pakistan, Peru, South Africa, Syria, Tuamotu Archipelago, Turkey (Domsch & al. 2007).

Habitat—Soil, stored and/or decaying food products among other substrates (Domsch & al. 2007, Pitt & Hocking 1997).

Comments—The morphological features of our strains agreed with those described by Thom & Raper (1941), Raper & Fennell (1965), Klich (2002) and Hubka & al. (2013). This species was also known as *Eurotium amstelodami* because Thom & Raper (1941) changed the original species

concept of Mangin (1909), from a species with smooth-walled ascospores to a species with ornamented ones. Their 1941 published name, *E. amstelodami* was, in fact, a later illegitimate homonym. Pitt (1985) noted this confusion and speculated that Thom & Church (1926) and Thom & Raper (1941) did not base their description on the Mangin species concept. Hubka & al. (2013) concluded that the first available and validly published name for the species matching the concept of Thom & Raper (1941) is *A. montevidensis*, which includes both the anamorph and the teleomorph states.

Aspergillus sydowii (Bainier & Sartory) Thom & Church, Aspergilli: 147. 1926.

Colonies on MEA, 25 °C, 7 d, velutinous to slightly floccose, 14–15 mm diam., blue green, asexual sporulation copious. On CYA, 25 °C, 7 d, sulcate, velutinous, 20 mm diam., blue green, exudate brown copious; asexual sporulation abundant; reverse orange brown. On 25% glycerol nitrate agar (G25N), 25 °C, 7 d, plane and dense, 11–13 mm diam., bluish grey.

Conidial heads radiate, biseriate; small and short penicillate heads produced. Stipes 250–350 µm, pigmented. Conidia globose, 3.5–4 µm, very rough to spinose. Hülle cells abundantly produced.

Cultures examined—**ARGENTINA. Catamarca**: **Rincón**, 28°22′03″S 66°13′39″W, 8.I.2009, leg. SMR, identified by SMR, isolation 4.VI.2009, (BAFCcult 4589); **RP 25**, 28°22′41″S 66°11′50″W, 1304 m a.s.l., 23.VIII.2011, leg. SMR, identified by SMR, isolation 10.II.2012 (BAFCcult 4612).

Distribution—Argentina, Australia, Austria, Brazil, British Isles, Canada, Czechoslovakia, Egypt, India, Italy, Japan, Kuwait, Malaysia, Namibia, Pakistan, Peru, Singapore, South Africa, Spain, Syria, ex USSR, U.S.A. (Domsch & al. 2007).

Habitat—Mainly isolated from soil, but also from air (indoor and outdoor) (Klich 2002). This species has been reported from numerous foods, especially dried ones (Pitt & Hocking 2009). It has been recovered also from marine sponges (Ein-Gil & al. 2009).

Comments—The features of our isolates matched those described by Raper & Fennell (1965) and Klich (2002). In Argentina, *A. sydowii* has been previously isolated from air (Winitzky 1948), amaranth seeds (Bresler & al. 1995), dried vine fruits (Romero & al. 2005), corn and soybean seeds (Sepúlveda & Piontelli 2005), soil (Allegrucci & al. 2007, Elíades & al. 2006a, Mangiaterra & al. 2006) and medicinal herbs (Sánchez & al. 2006). This is the first report for Catamarca province.

Epicoccum nigrum Link, Mag. Gesell. Naturf. Freunde Berlin 7: 32. 1816.

Colonies on Blakeslee's MEA, 25 °C, 7 d, floccose, 53–58 mm diam., orange brown, exudate and soluble pigment absent; reverse orange. On oatmeal agar (OA), 25 °C, 7 d, lanose to floccose, 60 mm diam., orange with yellowish centre, exudate absent, soluble pigment yellow; reverse yellowish orange; after 12 d of incubation brownish green centre due to sporulation. On PDA, 25 °C, 7 d, 23–30 mm diam., orange brown, exudate red caramel, soluble pigment amber; reverse red brown.

Conidiophores crowded, even forming pustules. Conidia globose to pyriform 22–30 × 18–29 µm, orange brown to dark brown, verrucose, several septa which divide the conidia in different directions, pale extending cell at the base.

Culture examined— **ARGENTINA. Catamarca**: **Papachacra**, 2564 m a.s.l., 10.I.2009, leg. SMR, identified by SMR, isolation 1.III.2011 (BAFCcult 4622).

Distribution—Worldwide (Domsch & al. 2007).

Habitat—Cereals and grains, fruits, soil, among others (Domsch & al. 2007).

Comments—The morphologic characteristics of our isolate agreed with the descriptions of *Epicoccum nigrum* by Ellis (1971) and Domsch & al. (2007). In Argentina there are numerous records of this species, which were isolated from soil samples of Jujuy province (Giusiano & al. 2002), wheat and soybean of Entre Ríos province (Broggi & al. 2007), soil and litter of Buenos Aires province (Allegrucci & al. 2005, 2007), and alpataco fruits from La Pampa province (Canafoglia & al. 2007), among others.

Gilmaniella humicola G.L. Barron, Mycologia 56: 514. 1964. Fig. 1P,Q

Colonies on MEA, 25 °C, 7 d, velutinous to slightly floccose, 59–75 mm diam., grayish, darkening in age; reverse very dark to black. At 30 °C, 7 d, velutinous to slightly floccose, 79–83 mm diam., grayish, darkening in age; reverse very dark to black.

Hyphae light brown in age, smooth. Conidia globose, terminal or lateral, usually in clusters and provided with a distinct germ pore, smooth, brown 7–9 µm.

Cultures examined—**ARGENTINA**. **Catamarca**: **RP 25**, 28°15′31″S 66°08′47″W, 1424 m a.s.l., 23.VIII.2011, leg. SMR, identified by SMR & RC, isolation 11.I.2012 (BAFCcult 4610, 4588).

Distribution—Canada, Egypt, England, France, India, Japan, Namibia, Solomon Islands, The Netherlands, United States of America (Domsch & al. 2007).

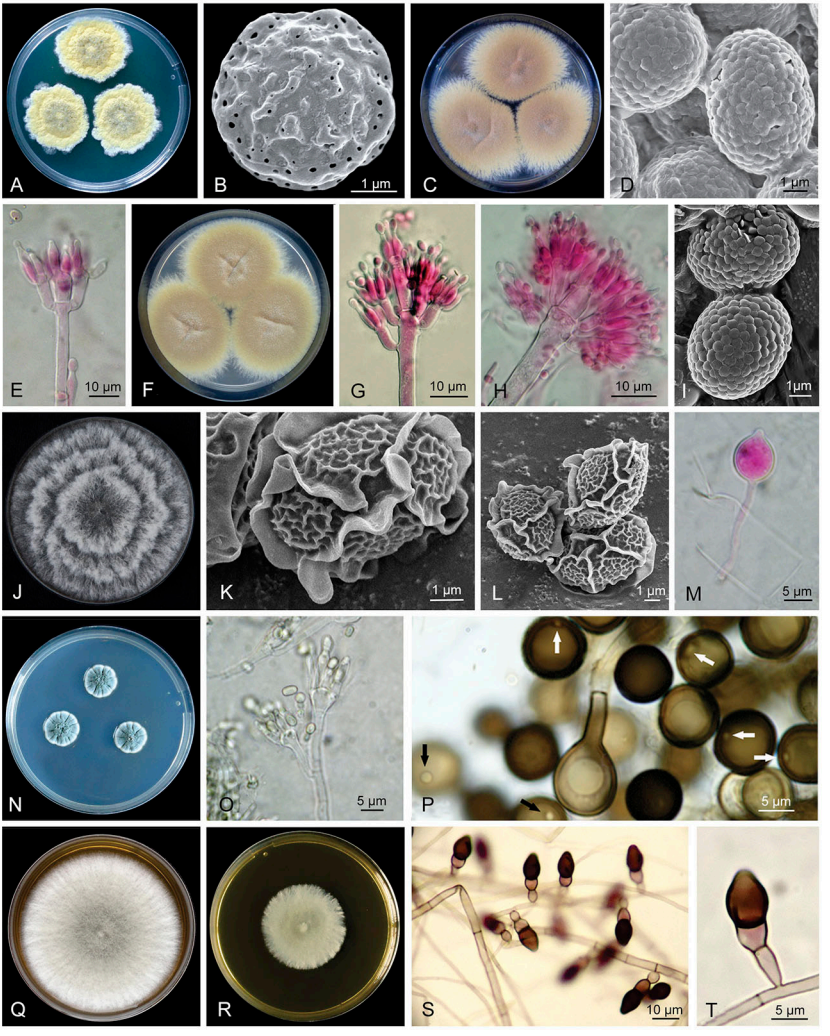

Fig. 1. ***Aspergillus montevidensis*** (BAFCcult 4613): A. Colonies on Czapek yeast extract agar (CYA) with 20% of sucrose, 14 d, 25 °C; B. Ascospore (SEM). ***Hamigera paravellanea*** (BAFCcult 4605): C. Colonies on CYA, 7 d, 25 °C; D. Ascospores (SEM); E. Conidiophore and conidia. ***Hamigera terricola*** (BAFCcult 4580): F. Colonies on CYA, 7 d, 25 °C; G–H. Conidiophores and conidia; I. Ascospores (SEM). ***Leiothecium ellipsoideum*** (BAFCcult 4598): J. Colony on MEA, 7 d, 25 °C; K, L. Ascospores (SEM); M. Chlamydospore. ***Penicillium capsulatum*** (BAFCcult 1627): N. Colonies on CYA, 7 d, 25 °C; O. Conidiophore and conidia. ***Gilmaniella humicola*** (BAFCcult 4588): P. Conidia with germination pores (arrows); Q. Colony on MEA 7 d, 30 °C. ***Trichocladium pyriforme*** (BAFCcult 4623): R. Colony on MEA 14 d, 25 °C; S, T. Chlamydospores.

Habitat—Dung, groundnuts, plant debris, soil, among others (Domsch & al. 2007).

Comments—Our isolates were phenotypically in accordance with Barron (1964) and Ellis (1971). *Gilmaniella humicola* is a good representative example of a heat-resistant fungus (Domsch & al. 2007), and it was the most thermal-resistant fungus found by Bollen (1969), tolerating a temperature of 90 °C for 30 minutes. All our isolates were recovered from samples collected in winter. In Argentina, the fungus was previously reported from soil in a *Nothofagus dombeyi* forest in Río Negro province (Godeas 1977).

Hamigera paravellanea S.W. Peterson, Jurjević, Bills, Stchigel, Guarro & F.E. Veja, Mycologia 102: 852. 2010. Fig. 1c–e

Colonies on MEA, 25 °C, 7 d, plane somewhat floccose, 47–67 mm diam., pale orange to tan; reverse dark, mainly at colony centre. On CYA, 25 °C, 7 d, low, slightly sulcate, 50–70 mm diam., pale orange to tan; reverse dark reddish-brown.

Gymnothecia globose, discrete, 120–280 µm diam., cream coloured, surrounded by a profuse hyphal net. Asci subglobose to claviform, 8-spored, 15–20 × 11–12 µm, evanescent. Ascospores broadly ellipsoidal, 7–7.5 × 5–6 µm, hyaline, thick-walled, echinulate. Conidiophores penicillate, 120–480 × 3–5 µm. Stipes slightly spathulate, smooth or roughened towards their bases, widest at their apices. Metulae with wide apices, 10–15 × 4–6 µm. Phialides ampulliform, 8–6 × 2–3 µm. Conidia ellipsoidal, 3–4 × 2–3 µm., smooth.

Cultures examined—**ARGENTINA. Catamarca: RP 25**, 28°15′31″S 66°08′47″W, 1424 m a.s.l., 6.I.2009, leg. SMR, identified by SMR, AMS & ERA, isolation 20.IX.2011 (BAFCcult 4605, FMR 16758; GenBank LT991984, LT991994); 23.VII.2011, leg. SMR, identified by SMR, AMS & ERA, isolation 11.I.2012 (BAFCcult 4606, FMR 16762; GenBank LT992033, LT992032).

Distribution—Poland, Spain (Peterson & al. 2010).

Habitat—Soil, dung (Peterson & al. 2010).

Comments—Morphological characters observed agreed with the description of the protologue (Peterson & al. 2010). This is the first report for Argentina.

Hamigera terricola S.W. Peterson, Jurjević, Bills, Stchigel, Guarro & F.E. Veja Mycologia 102: 855. 2010. Fig. 1f–i

Colonies on MEA, 25 °C, 7 d, low, sulcate, velutinous, 58–70 mm diam., ochraceous buff to tan; reverse dark reddish-brown. On CYA, 25 °C, 7 d, low,

plane, velutinous, 40–43 mm diam., ochraceous buff with yellowish margins; reverse dark reddish-brown.

Gymnothecia globose, discrete, 80–180 µm diam., cream coloured, surrounded by a profuse hyphal net. Asci subglobose, 8-spored, 15–17.5 × 11–13 µm, evanescent. Ascospores broadly ellipsoidal, 7–8 × 5–6 µm, hyaline, thick-walled, echinulate. Conidiophores penicillate, 170–400 × 3–6 µm. Stipes sometimes spathulate, with apical vesicles 7–9 µm wide, smooth or slightly roughened. Metulae with wide apices, 7–11 × 3–6 µm. Phialides ampulliform, 7–8 × 3 µm. Conidia ellipsoidal, 3–4 × 2–3 µm., smooth.

Culture examined—**ARGENTINA. Catamarca**: **RN 60**, km 1102, 28°55′15″S 66°08′46″W, 338 m a.s.l., 5.I.2009, leg. SMR, identified by SMR, AMS & ERA, isolation 4.VI.2011 (BAFCcult 4580, FMR 16756; GenBank LT991985, LT991995).

Distribution—Costa Rica, Equatorial Guinea, French Guiana, Guatemala, Panama (Peterson & al. 2010).

Habitat—Textile sample in contact with soil (Peterson & al. 2010).

Comments—In a broad sense, the features of this isolate matched with those described by Peterson & al. (2010), but ascospores are slightly larger and the asci smaller than in the original description. This is the first report of *Hamigera terricola* for Argentina.

Leiothecium ellipsoideum Samson & Mouch.,
Can. J. Bot. 53: 1634. 1975. Fig. 1J–M

Colonies on MEA, 25 °C, 7 d, forming concentric rings, 82–83 mm diam., dark, superficial white mycelium present; reverse dark. At 35 °C, covering the whole culture plate before 7 d; reverse dark due to ascomata abundance. On OA, 25 °C, 7 d, colonies presenting a continuous layer of ascomata, dark, almost lacking aerial mycelium. At 35 °C, covering the whole culture plate before 7 d; subtle annularly developed, less aerial mycelium than on MEA.

Vegetative hyphae smooth, 3–11 µm diam., hyaline. Cleistothecia globose, discrete, 40–90 µm diam., dark brown, superficial or semi-submerged in the substratum, covered by white mycelium supporting ascomata as well; wall persistent, textura angularis, 5–8 µm wide, composed by 15–25 µm diam. dark brown cells. Asci globose to subglobose, 14–18 × 13–15 µm, evanescent. Ascospores ellipsoidal, 7–9 × 5.5–7 µm, hyaline, spinulose under low magnifications, broadly reticulate under high magnifications; young ascospores presented an internal hyaline sheath which fades with maturity; SEM observations revealed alveolate plaques on ascospore wall, alveoles presented conspicuous projections at their edges which look as low frills.

Chlamydospores isolated, subglobose to ellipsoidal, umbonate, 4.5–11 µm diam., smooth, endogenous, and also terminal in maturity.

Cultures examined— **ARGENTINA. Catamarca: RN 60**, km 1146, 28°13'05"S 66°22'41"W, 1051 m a.s.l., 5.I.2009, leg. SMR, identified by SMR, RC & AMS, isolation 13.IX.2011 (BAFCcult 4598, FMR 15064; GenBank LT992254, LT992257); km 1016, 29°30′04″S 65°37′57″W, 237 m a.s.l., 22.VIII.2011, leg. SMR, identified by SMR, RC & AMS, isolation 12.XI.2011 (BAFCcult 4604, FMR 16765; GenBank LT992253, LT992256); **RP 47**, km 35.5, 27°26′50″S 66°24′26″W, 2700 m a.s.l., 24.VIII.2011, leg. SMR, identified by SMR, RC & AMS, isolation 18.I.2012 (BAFCcult 4609, FMR 16768; GenBank LT992255, LT992258).

Distribution—Canada (Samson & Mouchacca 1975), Greece, Hungary, Portugal, Spain, United States of America, and Venezuela (CBS-KNAW Culture Collection 2018).

Habitat—Soil, seeds of *Capsicum annuum* (Samson & Mouchacca 1975), nest material of *Nomia* sp., a ground-nesting solitary bee (CBS-KNAW Culture Collection 2018).

Comments—Argentinian isolates largely agreed with the description given by Samson & Mouchacca (1975) and Guarro & al. (2012). However, these strains grew faster on MEA at 35 °C than the ex-type strain. Regarding micromorphology, no chlamydospores in chains were observed (solitary chlamydospores were seen on small hyphal protrusions) and ascomata were smaller (40–90 µm vs. 125 µm diam) than those described in the protologue. This is the first report of *Leiothecium ellipsoideum* for Argentina.

Penicillium capsulatum Raper & Fennell, Mycologia 40: 528. 1948. Fig. 1n,o

Colonies on MEA, 25 °C, 7 d, low, plane, velutinous, 14–18 mm diam., white mycelium at the edges, grey green to dull green; conidiogenesis moderate; exudate and soluble pigment absent; reverse pale to yellowish. On CYA, 25 °C, 7 d, moderately deep, sulcate, slightly fasciculate, 12–15 mm diam.; conidiogenesis dense, grey green to dull green, white mycelium at the edges; exudate and soluble pigment absent; reverse pale to yellowish. At 37 °C, 7 d, funiculose, 18–21 mm diam., with white mycelium at the edges; exudate and soluble pigment absent; reverse pale to buff. At 5 °C germination absent. On G25N, 25 °C, 7 d, sulcate, velutinous to floccose, 6 mm diam., white mycelium at the edges, conidia in mass grey green to dull green; exudate and soluble pigment absent; reverse pale to yellowish.

Teleomorph absent. Conidiophore monoverticillate, arising from substrate surface or from superficial mycelium. Stipes somewhat sinuose, 10–40 µm long., smooth, mainly non-vesiculate, but sometimes vesicles up to

4 µm diam. Phialides 5–8, ampulliform to acerose, 6 × 2 µm, occasionally up to 8 µm in length, with short collula. Conidia ellipsoidal to cylindrical, 3–3.5 × 2 µm, smooth-walled.

Culture examined—**ARGENTINA. Catamarca: RP 33**, 28°42′03″S 65°46′08″W 418 m a.s.l., 22.VIII.2011, leg. SMR, identified by SMR & RC, isolation 23.XI.2011 (BAFCcult 1627).

Distribution—China (Chen & al. 2013), Kiribati (Gilbert Islands), Panamá (Raper & Fennell 1948).

Habitat—Optical instrument, environmentally exposed canvas and deteriorated military equipment (Raper & Fennell 1948), and contaminated beet pulp (Puls & Coughlan 1996).

Comments—Our soil-borne isolate matched the Pitt (1979) description. We also observed vesiculate conidiophores, but phialides were a bit shorter than in the holotype. Pitt (1979) described the colonies on CYA at 25 °C as depressed at the center, whereas the Argentinian strains presented a high and fasciculate center. This is the first report of *Penicillium capsulatum* for Argentina.

Penicillium citrinum Thom, U.S.D.A. Bur. Animal Industr. Bull. 118: 61. 1910.

Colonies on MEA, 25 °C, 7 d, low, plane, centrally umbonate, velutinous, 18–22 mm diam., grey green to dull green; conidiogenesis dense; exudate pale, soluble pigment absent; reverse pale. On CYA, 25 °C, 7 d, moderately deep, sulcate, velutinous, 24–30 mm diam., grey green to dull green; conidiogenesis dense, white mycelium at the edges; exudate pale, soluble pigment yellowish; reverse yellow to orange. At 37 °C, 7 d, 2–11 mm diam. At 5 °C no germination.

Teleomorph absent. Conidiophores biverticillate, arising from substrate surface or from superficial mycelium. Stipes with smooth walls and a conspicuous divaricate metulae whorl. Metulae of uniform length, 3–5 on each stipe. Phialides ampulliform, short collula. Conidia globose to subglobose, 2.5–3 µm diam., smooth-walled.

Culture examined—**ARGENTINA. Catamarca**: **Corral Quemado to Papachacra**, 27°07′33″S 66°56′36″W, 2152 m a.s.l.: 10.I.2009, leg. SMR, identified by SMR & RC, isolation 5.III.2011 (BAFCcult 4616).

Distribution—*Penicillium citrinum* is considered a cosmopolitan and frequently isolated fungus (Domsch & al. 2007).

Habitat—The fungus has been reported from many substrates, including food (Domsch & al. 2007).

Comments—Our isolate matched with the description of *Penicillium citrinum* provided by Pitt (1979). In Argentina, it has been isolated many times, generally

from food, e.g., soybeans (Bonera & al. 1982), amaranth seeds (Bresler & al. 1991, 1995), balanced feed for birds (Magnoli & al. 1998), and dried vine fruits (Romero & al. 2005), among others.

Talaromyces macrosporus (Stolk & Samson) Frisvad, Samson & Stolk, Antonie van Leeuwenhoek 57: 186, 1990.

Colonies on MEA, 25 °C, 14 d, almost covering the whole culture plate, moderate deep, plane, granulose, covering the whole culture plate, yellow, becoming orange at the centre.

Cleistothecia superficial, mainly globose, commonly confluent but at the margin discrete, 200–400 µm diam., yellow. Asci globose, 8-spored, evanescent; initials showing thin antheridia coiled around slender clavated ascogonia. Ascospores ovoidal to broadly ellipsoidal, 5.5–6.5 × 3.5–4 µm, pale yellow, with thick walls and spines. Anamorph absent.

Culture examined—**ARGENTINA. Catamarca: RP 33**, 28°42′03″S 65°46′08″W, 418 m a.s.l., 22.VII.2011, leg. SMR, identified by SMR & AIR, isolation 23.XI.2011 (BAFCcult 4617).

Distribution—Ghana, Japan, Korea, Malaysia, New Guinea, Panama, Poland, South Africa, The Netherlands, United States of America (CBS-KNAW Culture Collection 2018, Stolk & Samson 1972).

Habitat—Soil, canned apples (Stolk & Samson 1972), freshly harvested strawberries (Frisón & al. 2012).

Comments—Our isolate agreed with the description of *Talaromyces macrosporus* presented by Stolk & Samson (1972), who recognize two varieties: *T. flavus* var. *flavus* and *T. flavus* var. *macrosporus*. Regarding these varieties, Beuchat (1988) pointed out that the strains with small ascospores (*T. flavus* var. *flavus*) are less thermal resistant than those with larger ones (*T. flavus* var. *macrosporus*). Frisvad & al. (1990) raised them to species rank based on ascospore size, heat-resistance, and secondary metabolite production. In Argentina, the species has previously been recorded in soils either as *T. flavus* s.l. or as *T. flavus* var. *flavus* (Bertoni 1973, Elíades & al. 2006 a,b, Magnoli & al. 1998). Frisón & al. (2012) reported *T. macrosporus* on fresh strawberries.

Talaromyces pinophilus (Hedgc.) Samson, N. Yilmaz, Frisvad & Seifert, Stud. Mycol. 70: 176. 2011.

Colonies on MEA, 25 °C, 7 d, moderately deep, plane, floccose, 34–36 mm diam., green; conidiogenesis sparse, yellow mycelium present, almost dominating colony colour; exudate pale, soluble pigment absent; reverse

yellowish. On CYA, 25 °C, 7 d, moderately deep, plane, floccose, 19–20 mm diam., greenish yellow; conidiogenesis sparse, yellow mycelium present predominating in colony colour; exudate pale, soluble pigment absent; reverse brown. At 37 °C, 7 d, 24–26 mm diam., with white mycelium at the edges; exudate and soluble pigment absent; reverse buff. At 5 °C no germination. On G25N, 25 °C, 7 d, plane, floccose, 3–5 mm diam., white mycelium; sporulation absent; exudate pale, soluble pigment absent; reverse buff.

Teleomorph absent. Conidiophores biverticillate, arising from substrate surface and also from aerial hyphae. Stipes 100–150 µm long., smooth. Metulae in whorls mainly of 5 elements, 10–12 µm long. Phialides 5–8, acerose, 8–10 µm long., tapered. Conidia globose to subglobose, 3–4 µm diam., smooth-walled.

Culture examined—**ARGENTINA. Catamarca**: **Corral Quemado to Papachacra**, 27°07′33″S 66°56′36″W, 2152 m a.s.l., 10.I.2009, leg. SMR, identified by RC, isolation 5.III.2011 (BAFCcult 4618).

Distribution—Argentina (Magnoli & al. 1998); Australia, France, India, Papua New Guinea, United States of America (Pitt 1979); Uruguay (Galvalisi & al. 2012).

Habitat—Soil, decaying plants, deteriorated material, barley grains, fermented dry sausages, balanced feed for birds (references as above).

Comments—Excluding conidia, which in this case were a little bit bigger (in contrast to 2.5–2.8 × 2.2–2.5 µm), we did not find morphological differences compared to those of *Talaromyces pinophilus* given by Pitt (1979). In Argentina, it has been previously reported as *Penicillium pinophilum* from corn, balanced feed for birds, and soil (Etcheverry & al. 1999, Magnoli & al. 1998, Nesci & al. 2006, Mangiaterra & al. 2006).

Talaromyces trachyspermus var. ***macrocarpus*** J.E. Wright & Loewenb., Bol. Soc. Argent. Bot. 15(1): 100. 1973.

Colonies on MEA, 25 °C, 14 d, moderately deep, plane, granular, 37–44 mm diam., white to cream-colored; exudate absent; reverse orange.

Cleistothecia superficial, mainly globose, usually confluent, 400–930 µm diam., at first white becoming cream-colored in age, covered by a network of hyphal net. Asci globose to ovoid, 8-spored, 7–8 × 6–7 µm, evanescent. Ascospores ellipsoidal, 3–4 × 2–3 µm, spinulose. Conidiophores slender monoverticillate and biverticillate, short. Metulae 8–11 × 2 µm. Phialides 2–5 in verticils, lanceolate, tapering to neck, 9–10 × 2 µm. Conidia ellipsoidal, 2–2.5 × 2 µm, smooth-walled.

Culture examined—**ARGENTINA. Catamarca**: **RP 46**, km 185, 27°45′12″S 66°47′59″W, 1069 m a.s.l., 9.I.2009, leg. SMR, identified by SMR & AIR, isolation 21.III.2011 (BAFCcult 4621; GenBank LT968851).

Distribution—Argentina (Bertoni & al. 1973).

Habitat—Soil (Bertoni & al. 1973).

Comments—The characteristics of isolate BAFCcult 4621 agreed with the description of *Talaromyces trachyspermus* var. *macrocarpus* by Bertoni & al. (1973). This variety was not accepted by Pitt (1979); however, we prefer to recognize it until examination of the holotype, and study of more isolates and their molecular data. *T. trachyspermus* var. *macrocarpus* is distinguished from *T. trachyspermus* by presenting bigger cleistothecia (500–1500 vs 50–350 µm) and ascospores (4–4.5 × 2.8–3.2 vs 3–3.5 × 2–2.5 µm).

Talaromyces trachyspermus (Shear) Stolk & Samson, Stud. Mycol. 2: 32 (1973) var. ***trachyspermus***

Colonies on MEA, 25 °C, 14 d, moderately deep, plane, granular, 42–50 mm diam., white to cream-colored; exudate absent; reverse light orange and orange to brown in the centre.

Cleistothecia superficial, mainly globose, usually confluent, 150–350 µm diam., at first white becoming cream-colored in age, covered by a hyphal network. Asci globose to ovoid, 8-spored, 7–9 × 6–8 µm, evanescent. Ascospores ellipsoidal, 3.5–4 × 2–3 µm, spinulose. Conidiophores slender monoverticillate and biverticillate, short. Metulae 8–11 × 2 µm. Phialides 2–5 in verticils, lanceolate, tapering to neck, 9–10 × 2 µm. Conidia ellipsoidal, 2–2.5 × 2 µm, smooth-walled.

Cultures examined—**ARGENTINA. Catamarca**: **RP 46**, 27°35′13″S 66°22′11″W, 996 m a.s.l., 9.I.2009, leg. SMR, identified by SMR & AIR, isolation 21.III.2011 (BAFCcult 4619; GenBank LT968849); (BAFCcult 4620; GenBank LT968850); 28°46′22″S 66°09′56″W, 1317 m a.s.l., 24.VIII.2011, leg. SMR, identified by SMR & AIR, isolation 4.I.2012 (BAFCcult 4629); **Belén**, 27°07′33″S 66°56′36″W, 2152 m a.s.l., 25.VIII.2011, leg. SMR, identified by SMR, isolation 27.I.2012 (BAFCcult 4630); **RP 47**, km 35.5, 27°26′50″S 66°24′26″W, 2700 m a.s.l., 25.VIII.2011, leg. SMR, identified by SMR, isolation 18.I.2012 (BAFCcult 4631).

Distribution—Argentina, Bangladesh, France, Germany, India, Japan, Nepal, Pakistan, South Africa, Tahiti, Uganda, United States of America (Domsch & al. 2007).

Habitat—Soil, cereals and grains, dung (Domsch & al. 2007).

Comments—The morphological features of our isolates agreed with the original description of *Talaromyces trachyspermus* (Stolk & Samson 1972). In

Argentina Bertoni & al. (1973) and Godeas (1975) isolated this fungus from soil.

Trichocladium pyriforme M. Dixon,
Trans. Br. Mycol. Soc. 51: 160. 1968. Fig. 1r–t

Colonies on MEA, a 25 °C, 7 d, plane, slightly floccose, 36–40 mm diam., grey green to olive, darkening in age; reverse very dark; covering the whole culture plate in 21 d.

Hyphae hyaline when young, yellow brown in age, 2–5 µm wide, smooth. Chlamydospores (holothallic conidia) terminal, pyriform, 3-4 celled, 16–19 × 6–7.5 µm, smooth-walled; distal cell dark brown, with an acute apex and germinative pore.

Cultures examined—**ARGENTINA. Catamarca: RN 60**, km 934, 29°33′35″S 64°52′56″W, 186 m a.s.l., 5.I.2009, leg. SMR, identified by SMR & AIR, isolation 5.I.2011 (BAFCcult 4623); **RN 40**, km 4243.5, 26°51′23″S 66°05′52″W, 2031 m a.s.l., 25.VIII.2011, leg. SMR, identified by SMR & AIR, isolation 12.XI.2011 (BAFCcult 4611).

Distribution—Czech Republic (Mantle & al. 2006), Ireland (Dixon 1968).

Habitat—Soil (Dixon 1968, Goh & Hyde 1999).

Comments—The morphological features of the Argentinian strains of *Trichocladium pyriforme* agreed with those described by Dixon (1968) and by Goh & Hyde (1999). Domsch & al. (2007) pointed out the remarkable thermal resistance of the spores of this species, and Dixon (1968) stated that it tolerated up to 95 °C for 10 min. Bollen (1969) isolated *T. pyriforme* from greenhouse soil using a thermal shock at temperatures greater than 55 °C. This is the first report of this fungus in Argentina.

Trichoderma saturnisporum Hammill, Mycologia 62: 112. 1970.

Colonies on special nutrient agar (SNA), 72 h, 30 °C, in darkness, somewhat annellated, 38–44 mm diam., green. At 35 °C, 72 h, 38 mm diam. On PDA, 72 h, 30 °C, slightly annellated, 60 mm, dull green; at 35 °C, 69 mm diam. in darkness.

Conidiophores associated in tufts up to 4 mm diam., branched, short sterile projections present. Phialides mainly in verticils, cylindrical, 8.5–11 × 3.3–4 µm. Conidia subglobose to broadly ellipsoidal, 4–4.8 × 2.9–3.7 µm, greenish, walls with few notorious warts. Chlamydospores not observed.

Cultures examined—**ARGENTINA. Catamarca: RN 60,** km 1016, 29°30′04″S 65°37′57″W, 237 m a.s.l., 5.I.2009, leg. SMR, identified by SMR & RC, isolation 3.VIII.2010 (BAFCcult 4584, 4586, 4587); **RP 25**, 28°14′36″S 66°08′51″W, 1507 m a.s.l., 6.I.2009, leg. SMR, identified by SMR & VB, isolation 1.XI.2010 (BAFCcult 4626);

Rincón, 28°22′03″S 66°13′39″W, 8.I.2009, leg. SMR, identified by SMR & VB, isolation 19.VIII.2010 (BAFCcult 4627, 4628.

Distribution—Argentina (Godeas & al. 1977), Australia, Italy, South Africa, Turkey, United States (Samuels & al. 1998).

Habitat—Soil.

Comments—The morphological features of the examined culture agreed with those given by Samuels & al. (1998). *Trichoderma saturnisporum* is easily recognized by conidia with large warts mimicking wings, which give a saturnian appearance to the conidia.

Discussion

In previous papers we described two new species of *Eurotiales*, *Talaromyces systylus* S.M. Romero & al. (Romero & al. 2016) and *Aspergillus fuscicans* S.M. Romero & al. (Romero & al. 2018) from the same locations and substrate (soil) as in the present work. The isolates in *Aspergillus* sect. *Fumigati*, which were prevalent in the analyzed samples, are currently under study.

Some of the fungal species found have been previously cited as heat-resistant, such as *Arthrinium phaeospermum* (Kashiwagi & al. 2009, Pitt & Hocking 2009), *Gilmaniella humicola* (Bollen 1969, Jesenská & al. 1992), *Leiothecium ellipsoideum* (Samson & Mouchacca 1975), *Talaromyces macrosporus* (Beuchat 1988, Frisón & al. 2012) and *Trichocladium pyriforme* (Bollen 1969, Dixon 1968).

It was not possible to obtain information about the heat-resistance of *Aspergillus montevidensis*; however, *Aspergillus glaucus* (L.) Link, another species in *A.* sect. *Aspergillus*, was isolated from contaminated jellies and grape marmalades, and its thermal resistance was analyzed (Splittstoesser & al. 1989). No information about the heat resistance was found for *Trichoderma saturnisporum*; however, chlamydospore production (Samuels & al. 1998) would explain its survival after a thermal shock performed in the lab.

Byssochlamys nivea, perhaps the most well-known heat-resistant fungal species, was not isolated during this study. This could be attributed to our thermal treatment (75 °C, 30 min), since other authors have determined that its ascospores can survive at 70 °C for 60–75 min but not at 80 °C for 1 min (Piecková & al. 1994). The same authors also mentioned that at 80 °C the ascospores of *Hamigera avellanea* Stolk & Samson survive 120 min, whereas the conidia of *Gilmaniella humicola* survive between 8–14 min. Although no data were found on the thermal resistance of *H. paravellanea* and *H. terricola* ascospores, given the heat resistance described for *H. avellanea* (Piecková & al.

1994, Scaramuzza & Berni 2014), it can be assumed that other species of the genera would possess a similar capacity.

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